

Amendment to the Claims:

Please amend the claims as follows, without prejudice or disclaimer:

Please cancel claims 2 to 17, 21, 35, 36, 38, and 40 without prejudice or disclaimer.

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A method for the preparation of simvastatin comprising

(i) a method as set forth in Figure 5, Figure 6A or Figure 38;

(ii) (a) an enzymatic hydrolysis of lovastatin, lovastatin acid or a salt of lovastatin acid to form a triol acid or a salt of a triol acid; (b) lactonization and acylation of the triol acid to form a 4-acetyl lactone, wherein the acylation comprises protecting a 4-position hydroxyl (4'-OH) on the lactone ring by regioselective acylation of the 4'-OH; (c) enzymatic acylation of an 8-position hydroxyl (8'-OH) of the 4-acetyl lactone to form a 4-acetyl simvastatin; and (d) removing selectively the acyl protecting group at the 4' position either chemically or enzymatically, thereby yielding simvastatin;

(iii) the method of (ii), wherein in step (b) the acylation comprises protecting a 4-position hydroxyl (4'-OH) on the lactone ring by enzymatic regioselective acylation of the 4'-OH;

(iv) the method of (ii), wherein in step (c) the enzymatic acylation of an 8-position hydroxyl (8'-OH) of the 4-acetyl lactone enzymatic regioselective acylation of the 8-position to form a 4-acetyl simvastatin;

(v) a homodiacylation process for the preparation of simvastatin comprising: (a) enzymatic hydrolysis of lovastatin, lovastatin acid or a salt of lovastatin acid to form a triol acid; (b) forming a diol lactone from the triol acid by lactonization; (c) acylating the 4-position (4'-OH) and 8-position (8'-OH) on the lactone ring of the diol lactone by chemical acylation to form a 4,8-diacetyl lactone; and (d) removing selectively the acyl group at the 4' position by enzymatic hydrolysis, thereby making simvastatin;

(vi) the method of any of (i) to (v), wherein at least one step is performed in a separate reaction vessel;

(vii) the method of (vi), wherein at least two steps are performed in separate reaction vessels;

(viii) the method of any of (i) to (vii), wherein at least one step is performed with a cell extract, or at least one step is performed in a whole cell;

(ix) the method of any of (i) to (viii), further comprising crystallization of the simvastatin;

(x) the method of (ix), further comprising re-crystallization of the simvastatin;

(xi) the method of any of (i) to (x), further comprising re-lactonization to provide simvastatin with a desired purity;

(xii) the method of any of (i) to (xi), wherein at least one enzymatic reaction is carried out by a hydrolase:

(a) encoded by a nucleic acid having at least 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:1, or enzymatically active fragments thereof;

(b) encoded by a nucleic acid having at least 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:3, or enzymatically active fragments thereof;

(c) encoded by a nucleic acid having at least 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:5, or enzymatically active fragments thereof; or,

(d) having a sequence at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6, or enzymatically active fragments thereof; or,

(xiii) the method of any of (i) to (xii), wherein the method comprises enzymatic hydrolysis of lovastatin to make a triol acid or a salt of a triol acid, followed by lactonization of the triol acid and enzymatic acylation of the 4-position (4'-OH) of the lactone ring to make a 4-acyl lactone, followed by enzymatic acylation of the 4-acyl lactone to make a 4-acetyl-simvastatin, followed by regioselective enzymatic hydrolysis of the 4-acetyl-simvastatin to make simvastatin.

Claims 2 to 17 (canceled)

18. (original): A method for preparing 4-acetyl lactone comprising enzymatic hydrolysis of lovastatin to make a triol acid or a salt of a triol acid, followed by lactonization of the triol acid to make a diol lactone, followed by regioselective enzymatic acylation of the diol lactone on the 4-position (4'-OH) of the lactone ring to make 4-acetyl lactone.

19. (original): A method for preparing 4-acetyl-simvastatin comprising enzymatic hydrolysis of lovastatin to make a triol acid or a salt of a triol acid, followed by lactonization of the triol acid to make a diol lactone, followed by regioselective enzymatic acylation of the diol lactone on the 4-position (4'-OH) of the lactone ring to make 4-acetyl lactone, followed by

regioselective enzymatic acylation of the 4-acetyl lactone on the 8-position (8'-OH) of the lactone make 4-acetyl-simvastatin.

20. (original): A method for the preparation of a triol acid or a salt of a triol acid from lovastatin comprising:

**(i)** (a) providing a lovastatin, lovastatin or a salt of lovastatin, and an esterase enzyme;

(b) contacting the lovastatin, lovastatin or a salt of lovastatin with the esterase under conditions wherein the esterase catalyzes the hydrolysis of the lovastatin to a triol acid or a salt of a triol acid; or,

**(ii)** the method of (i), wherein the esterase has a sequence at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6.

Claim 21 (canceled)

Claim 22 (original): A method for preparing a triol acid or a salt of a triol acid from a lovastatin comprising a method as set forth in Figure 15A, Figure 16A, Figure 18E or Figure 19.

Claim 23 (original): A method for preparing a triol acid from lovastatin acid comprising a method as set forth in Figure 16A.

Claim 24 (original): A method for preparing a lovastatin acid from a lovastatin comprising a method as set forth in Figure 16A.

Claim 25 (original): A method for preparing a diol lactone from a triol acid comprising a method as set forth in Figure 8.

Claim 26 (original): A method for preparing an acyl lactone from a diol lactone comprising a method as set forth in Figure 16C.

Claim 27 (original): A method for preparing an acyl lactone from a triol acid comprising a method as set forth in Figure 16D.

Claim 28 (original): A method for preparing a 4-acetyllactone from a triol acid comprising a method as set forth in Figure 9A.

Claim 29 (original): A method for preparing an acyl simvastatin from an acyl lactone comprising a method as set forth in Figure 16E.

Claim 30 (original): A method for preparing a 4-acetylsimvastatin from a 4-acetyllactone comprising a method as set forth in Figure 9B.

Claim 31 (original): A method for preparing a simvastatin from a 4-acetylsimvastatin comprising a method as set forth in Figure 9C or Figure 11.

Claim 32 (original): A method for preparing a simvastatin ammonium salt from an acyl simvastatin comprising a method as set forth in Figure 16F.

Claim 33 (original): A method for preparing a simvastatin from a simvastatin ammonium salt comprising a method as set forth in Figure 16F.

Claim 34 (currently amended): A method for preparing a simvastatin or related compound from lovastatin, a triol acid, a 4-acyl lactone or a 4-acyl simvastatin, comprising a method as set forth in Figure 5, Figure 6A or Figure 38, wherein the 4-position protecting group added in step 3 is a R- group selected from the group consisting of

(a) (i) - H, -methyl, or a formyl derivative; (ii) a C1-n alkyl, both straight chain and branched, wherein n is an integer between 1 and 20; (iii) a substituted alkyl group; (iv) phenyl and substituted phenyl: e.g., phenyl, p-nitrophenyl; and (v) an R'O- group, forming a carbonate protecting group, wherein R' is any group of (i), (ii), (iii) or (iv);

(b) the method of (a), wherein the substituted alkyl group comprises a chloroacetyl, a trichloroacetyl, a trifluoroacetyl, a methoxyacetyl, a phenylacetyl, a 4-oxopentyl (levulinate) or an equivalent thereof; or,

(c) the method of (a), wherein the carbonate protecting group comprises tBuOCO, PhOCO, PhCH<sub>2</sub>OCO or an equivalent thereof.

Claims 35 and 36 (canceled)

Claim 37 (currently amended): A kit comprising (a) reagents and at least one hydrolase enzyme for practicing the methods of claim 1, claim 2 or claim 5; and (b) the kit of (a), wherein the at least one hydrolase enzyme has a sequence having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6, or enzymatically active fragments thereof.

Claim 38 (canceled)

Claim 39 (original): A method for preparing simvastatin comprising a five-step heterodiacylation process having the following steps:

- (i) (a) enzymatic hydrolysis of lovastatin, lovastatin acid or a salt of lovastatin acid to form a triol acid or a salt of a triol acid;
- (b) lactonization of the triol acid to form a diol lactone;
- (c) protecting the hydroxyl at the 4-position (4'-OH) on the lactone ring of the diol lactone by enzymatic regioselective acylation of the 4'-OH to form a 4-acyl lactone;
- (d) acylating the hydroxyl at the 8-position (8'-OH) of the 4-acyl lactone by enzymatic regioselective acylation of the 8-position to form a 4-acyl simvastatin; and
- (e) removing selectively the acyl protecting group at the 4' position either chemically or enzymatically, thereby yielding simvastatin; or

(ii) the method of (i), wherein in step (b) the lactonization of the triol acid to form a diol lactone comprises heating the triol acid or stirring in the presence of acid to form a diol lactone.

Claim 40 (canceled)